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Forensic Exploitation of Veterinary Entomology

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Abstract

Forensic entomology is the study of the insects associated with an animal carcass in an effort to determine elapsed time since death. It was first reported to have been used in 13th Century in China. The life cycle of insects invading the carcass and their larval development pattern may help to assess the time of death. There are two ways of using insects to determine elapsed time since death: Successional waves of insects and maggot age and their development. The insect eggs are laid in batches on the carcass which hatch into a first instar, followed by a second instar and then a third instar larva. The developmental stages in the life span of insects takes a set known time. This time period is based on the availability of food and the temperature. The two main insect groups on carcass are flies (Diptera) and beetles (Coleoptera). Both types of insect look very different at different stages of their lives and can be identified.

Key words: Forensic Entomology, Diptera, Coleoptera, Time of death

Introduction

Forensic entomology is the study of the insects associated with an animal carcass in an effort to determine elapsed time since death. The life cycle of insects invading the carcass and their larval development pattern may help to asses the time of death. Insect evidence may also show that the body has been moved to a second site after death, or that the carcass has been disturbed at some time, either by animals, or by the human returning to the scene of the crime[1]. However, the primary purpose of forensic entomology is to know elapsed time since death. Forensic entomology was first reported to have been used in 13th Century China and was used sporadically in the 19th Century and the early part of the 20th Century, playing a part in some very major cases[2]. However, in the last 15 years, forensic entomology has become more and more common in investigations. Most cases that involve a forensic entomologist are 72hrs or more old, other forensic methods are equally or more accurate than the insect evidence. However, after three days, insect evidence is often the most accurate and sometimes the only method of determining elapsed time since death[3].

Procedure

The first and most important stage of the procedure involved in forensic entomology involves careful and accurate collection of insect evidence at the scene. This involves knowledge of the insect's behavior; therefore it is best performed by an entomologist. Unfortunately, the entomologist is often not called until after the body has been removed from the death site[9].

Collecting, Preserving and Packaging of Specimens:

Samples of insects of all stages should be collected from different areas of the body, from the clothing and from the soil/carpet *etc*. Insects will often congregate in wounds and in

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and around natural orifices. The two main insect groups on carcass are flies (Diptera) and beetles (Coleoptera). Both types of insect look very different at different stages of their lives. Flies can be found as eggs (in egg masses usually), larvae or maggots (in a range of sizes from 1-2 mm to 17 mm), pupae and/or puparium and adults.

Insect fauna associated with carcasses / forensic entomology in India

Order	Family	Species
Diptera	Calliphoridae	*Chrysomya megacephala* *Chrysomya rufifacies*
	Sarcophagidae	*Sarcophaga albiceps* *Sarcophagahirtipes*
	Muscidae	*Musca domestica nebulo* *Musca sorbens* *Musca pattoni*
	Anthomyiidae	*Adia cincerella*
	Otitidae	*Physiophora flaviceps*
Coleoptera	Histeridae	*Saprinus* sp. *Euspilotus* sp.
	Tenebrionidae	*Gonocephalum* sp. *Scleron reitteri*
	Dermestidae	*Dermestes maculatus*
	Staphylinidae	*Philonthus longicornis*
	Cleridae	*Necrobia* sp.
	Siphidae	*Calosilpha cyaniventris*
Hymenoptera	Formicidae	*Paratrechina longicornis* *Tapinoma melanocephalum* *Camponotus compressus*
Lepidoptera	Tineidae	*Monopis* sp.

Eggs

Eggs are very tiny, but are usually laid in clumps or masses, and are usually found in a wound or natural orifice, but may be found on clothing *etc*. They can be collected with forceps. Half should be preserved in 75% alcohol or 50% isopropyl alcohol. The rest should be placed in a vial with a little damp tissue paper to prevent dehydration. If it will be more than a few hours before the entomologist receives them, they should also be given a small piece of beef liver. Make sure there is tissue or sawdust present if liver is added, to prevent drowning. They need some air. Newly emerged maggots can escape through holes, so a paper towel held over the top of the vial with a rubber band is excellent, as long as the vial stays upright[10].

Maggots

Collect maggots of different sizes. Maggots will be found crawling on or near the remains and may be in maggot masses. The masses generate a lot of heat, which speeds up development. Therefore it is important to note the site, size, number and temperature of maggot masses.

Large maggots are usually older so are most important, but smaller maggots may belong to a different species so both large and smaller maggots should be collected, with the emphasis on larger maggots. Collect samples of maggots from different areas of the body and the surrounding area, and keep them separate.

As mentioned before, third in star larvae leave the food source to find a suitable area in which to pupate. They may wander some distance from the body so the soil for a metre or two around the body should be carefully sifted. Some may burrow down into leaf litter, so the soil below the carsass should be checked for several centimetres. If the remains were on a slope, the body fluids will seep downhill and insects will be found here, feeding on the fluids. This means that a very intensive search of the carcass, the clothes and the surrounding area must be made in order to get the entire picture[11].

When collected, a proportion of the larvae should be preserved immediately for two reasons. Firstly, to show the entomologist, if s/he is not present at the scene, what stage the larvae were in, when collected, as if they are then placed on meat, they will continue to develop, giving a misleading impression to the entomologist when they are examined. Secondly, to produce an evidence in court. If there are lots of maggots on the body, preserve approximately half of all sizes. If there are only 20-30, preserve 1 or 2[12]. Preserve the specimens by immersing them in hot water for a few minutes, then putting them in 70% alcohol or 50% isopropyl alcohol. Don't forget that most should be kept alive. A sample should contain about 100 maggots (of each size if possible). The living specimens should be placed in a vial, with air and food, as for the eggs[5].

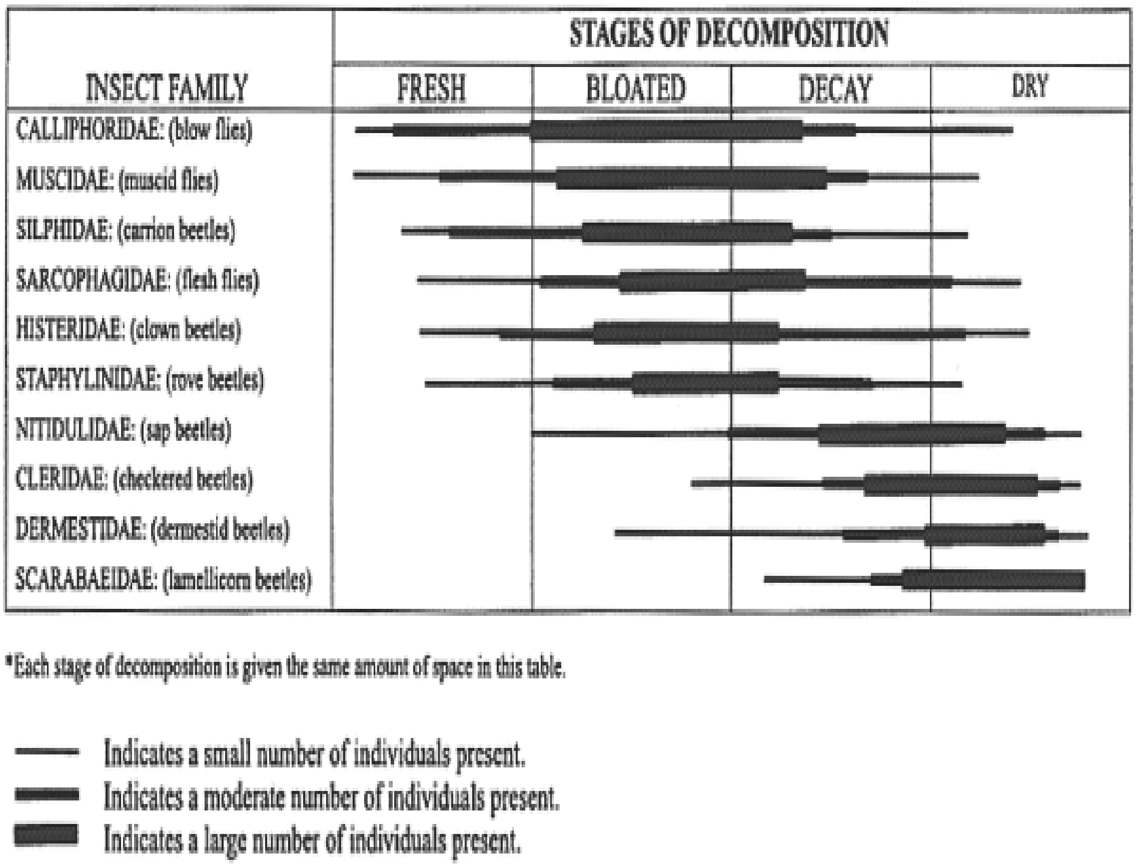
Pupae and Puparium

These are extremely important and are easy to miss. They are often found in clothing, hair or soil near the carcass. Pupae like dry, secure areas away from the wet food source to pupate. If the remains are found indoors, they may have traveled some distance and be under clothing, rugs, boxes *etc*. They range from 2-20 mm, and are oval, like a football. They are dark brown when completely tanned. An empty pupal case is very similar but is open at one end, where the adult fly has emerged. They need some air, so secure a paper towel over vial as for eggs, as although the pupae are immobile, if they emerge during transit, an adult can get

out of anything! A piece of tissue in the vial will help to avoid breakage as they are quite vulnerable. This can be *very slightly* moistened with water, but be careful not to drown them. The moisture isn't necessary if the journey is not long. Do not preserve pupae! They won't grow, so the reasons for preserving larvae do not apply, and it is almost impossible to identify a pupa until it emerges as an adult[5].

Adult Flies

Adult flies are less important. Adult flies are only of use in indicating which species of insect are likely to develop from the carcass, as it is difficult to determine whether an adult has developed on the carcass, or has just arrived from somewhere else to oviposit. If an adult



has crumpled wings, it may have just emerged, so is still important as it can be linked to the body. It should be collected, labeled as such and kept separate. Adults can be collected by net or by using an inverted vial. They can be left in the vial without air or food. This can be very important as it indicates that at least one generation of flies of this species has completed development on the carcass[5].

Beetles

Beetles can be found as adults, larvae, pupae and also as cast skins. All stages are equally important. They move fast and are often found under the carcass, and in and under bedding. They can be placed in vials with some air. They only need to be fed if it will be more than 24h before they reach an entomologist. If necessary they can be fed extra maggots. They are cannibals so should not be placed in the same vial[13].

Other Insects and samples: Other insects may be present. If you are not sure whether it's an insect, collect it anyway and place in a vial. Soil and leaf litter samples will also be useful. About a coffee can size of soil from under of very near the body is useful. If the soil below the body is extremely wet, it is better to collect the soil from near the remains.

Insects and their instars as forensic evidence

There are two ways of using insects to determine elapsed time since death; Successional waves of insects and maggot age and development. In general, the first method is used when the carcass has been dead between a month up to a year or more, and the second method is used when death occurred less than a month. The first method is based on the fact that an animal carcass, or any kind of carrion, supports a very rapidly changing ecosystem going from the fresh state to dry bones in a matter of weeks or months depending on geographic region. During this decomposition, the remains go through rapid physical, biological and chemical changes, and different stages of the decomposition are attractive to different species of insects. Certain species of insects are often the first witnesses to a crime. They usually arrive within 24hrs of death if the season is suitable *i.e.* spring, summer or fall and can arrive within minutes in the presence of blood or other body fluids. These first groups of insects are the Calliphoridae or blowflies and the Muscidae or houseflies. Few species are not attracted towards the fresh carcass, but are only attracted to the carcass later during protein fermentation such as the Piophilidae or cheese skippers. Some insects are not attracted towards carcass directly, but arrive to feed on the other insects at the scene. Many species are involved at each decomposition stage and each group of insects overlaps the ones adjacent to it somewhat. Therefore, with knowledge of the regional insect fauna and times of carrion colonization, the insect assemblage associated with the remains can be analyzed to determine the time in which death took place. It can also be used to indicate the season of death *e.g.* early summer. Knowledge of insect succession is required for this method to be successful[4].

The second method i.e using maggot age and development can give a date of death accurate to a day or less, or a range of days, and is used in the first few weeks after death. The insects used in this method are those that arrive first on the carcass, that is, the Calliphoridae or blowflies. These flies are attracted to a carcass very soon after death. They lay their eggs on the carcass, usually in a wound, if present, or if not, then in any of the natural orifices. Their development follows a set, predictable, cycle[5]. The insect egg is laid in batches on the carcass and hatches, after a set period of time, into a first instar (or stage) larva. The larva feeds on the carcass and moults into a second instar larva. The larva continues to feed and develop into a third instar larva. The stage can be determined by size and the number of spiracles (breathing holes). When in the third instar, the larva continues to feed for a while then it stops feeding and wanders away from the carcass, either into the clothes or the soil, to find a safe place to pupate. This non-feeding wandering stage is called a prepupa. The larva then loosens itself from its outer skin, but remains inside. This outer shell hardens, or tans,

into a hard protective outer shell, or pupal case, which shields the insect as it metamorphoses into an adult. Freshly formed pupae are pale in colour, but darken to a deep brown in a few hours. After a number of days, an adult fly will emerge from the pupa and the cycle will begin again. When the adult has emerged, the empty pupal case is left behind as evidence that a fly developed and emerged[6].

The developmental stages in the life span of an insect take a set known time. This time period is based on the availability of food and the temperature. In the case of a animal carcass, food availability is not usually a limiting factor, hence their development is extremely temperature dependent. Their metabolic rate is increased with increased temperature, which results in a faster rate of development, so that the duration of development decreases in a linear manner with increased temperature, and *vice-versa*[7].An analysis of the oldest stage of insect on the carcass and the temperature of the region in which the body was discovered leads to a day or range of days in which the first insects oviposited or laid eggs on the corpse. This, in turn, leads to a day, or range of days, during which death occurred. For example, if the oldest insects are 7 days old, then the decedent has been dead for at least 7 days. This method can be used until the first adults begin to emerge, after which it is not possible to determine which generation is present. Therefore, after a single generation of insects has been completed, the time of death is determined using the first method, that of insect succession[8].

Approximate life cycle of different Insects of forensic importance

Insects	**Egg Hrs.**	**1st instar Hrs.**	**2nd instar Hrs.**	**3rd instar Hrs.**	**Prepupa Hrs.**	**Pupa Hrs.**	**Total time (days)**
Lucilia	20-24	30-35	20-30	45-55	115-120	240-250	18-25
Diptera	20-24	35-40	35-40	70-80	70-80	160-170	15-20
Musca	8-20	60-80	60-100	60-100	65-85	90-110	9-18
Calliphoridae	40-42	50-55	40-45	95-99	140-150	393	30-35
Culiciodes	1 day	1 day	1 day	2 days	4 days	10 days	19-22
Drosophila	1 day	1 day	1 day	1 day	1-3 days	3-5days	8-12
Beetles	23-54	4-5 days	5-6 days	5-6 days	10-12 days	3-7 days	28-38

Labeling, handling and packing

Insects collected from one part of the body should be kept separate from those from another area. Different species should be kept separate as beetle larvae feed on fly larvae! If they look different, separate them. Each vial should be labeled according to the area of carcass/soil, date and time of collection, name of collector and stage of insect e.g. larvae. Most specimens are fairly fragile and are probably best picked up with gloved fingers which are often gentler than forceps if you are not used to them. Very tiny or delicate specimens can be picked up using an artist's brush dipped in water or alcohol depending on what you are about to do with them. Make sure all the vials are very well sealed. The insects should be taken to the entomologist as soon as possible. They should be couriered or hand delivered to

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maintain continuity. They should be packaged in a cardboard box as this has lots of air. Each vial can be taped so that it remains upright. The whole box must remain upright.

One needs to know many other factors about the death site:-

If the carcass is refrigerated before the collection (it is much better to collect at the scene, but sometimes that is not possible) then one should know the exact time that the carcass went into the cooler, and the exact time it came out. Photographs- or a video of the scene, the body *in situe* and the site after removal of the body are also extremely useful.

When the insects reach the insectary, the immature specimens are measured, and examined, then placed in a jar containing a suitable feeding media. In the case of blowflies, this is usually beef liver, which is placed on top of sawdust. When the insects reach the prepupal stage and leave the food source they will burrow into the sawdust to pupate. The insects are checked daily and when they pupate they are removed and placed in a petridish with damp filter paper. The date of pupation and the date of emergence is noted for each specimen. When the adults emerge, they are killed and pinned, then placed in an insect box. Each insect has a detailed label. Any adults collected directly from the carcass are immediately killed and pinned[5]. The reasons for raising the immatures are two-fold. Firstly, larvae are very difficult to identify to species, but adults have many more diagnostic features. Secondly, the dates of pupation and emergence are used to help calculate the age at the time of collection. Other important information used to determine elapsed time since death include weather records from the nearest weather station, including temperature and precipitation and the distance between the death site and the weather station.

Malpractices amounting to destroying the entomological evidences

The carcass may have been moved after death, from the scene of the death to a hiding place. Some of the insects on the body may be native to the first habitat and not the second. This will show that not only was the body moved, but it will also give an indication of the type of area where the death actually took place[14]. The carcass may have been disturbed after death, by the human returning to the scene of the crime. This may disturb the insects cycle, and the entomologist may be able to determine not only the date of death, but also the date of the return of the human to the death site[15].

The presence and position of wounds, decomposition may obscure wounds. Insects colonize remains in a specific pattern, usually laying eggs first in the facial orifices, unless there are wounds, in which case they will colonize these first, then proceed down the carcass. If the maggot activity is centered away from the natural orifices, then it is likely that this is the site of a wound. For example, maggot activity on the palm of the hands indicates the probable presence of defense wounds[16]. The presence of drugs can be determined using insect evidence. Sometimes there is not enough flesh left on the carcass to determine drug presence, but maggot's bioaccumulation can be analyzed to determine type of drug present[17].

Limitations

1. The temperature of the death site is obviously a very important factor, but in few cases animal carcasses were found underneath a weather station. In most cases, the weather records come from several miles away. We are trying to overcome this by setting up a miniature weather station at the death site after discovery, to compare these data with that from the weather station, in order to determine the difference between the two sites, if any. Also the microclimate of the carcass itself will be slightly different from the

surrounding area, especially if a maggot mass is present. Therefore, it is extremely important to know whether masses are present[18].

2. Forensic entomology is seasonal, that is, it is commonly used in spring, summer, and fall when insects are abundant. It is of less use in winter, unless it's very mild, as there are no or very few insects present. This can be a limitation
3. The results are not immediate, as it takes time to rear the insects. DNA evidence is now being developed to speed up identification of immature specimens.
4. The carcass may have been disposed of in a way that excludes insects *e.g.*

(a) Freezing - if the carcass was frozen for a period of time before being placed outside, the insects would only invade then, giving the misleading impression about the time of death. However, other forensic experts would be able to determine whether or not the carcass has been frozen, and insect evidence will still determine time of exposure.

(b) Burial - if the carcass is buried deeply then most insects will be excluded. However, most of the burials are not very deep, as the aim is merely to conceal the carcass and most insects will dig down to the carcass, particularly if there is blood soaked in the soil. Therefore, insect evidence can still be used.

(c) Wrapped - if the carcass is wrapped or packaged in such a way that the insects may be excluded. A body part was found sealed in a garbage bag which had been tied securely at the top, but the remains were maggot-infested and showed severe insect damage. The adult females had probably laid their eggs at the knot and the first instar larvae had crawled in.

5. More research is needed. Insect succession varies from geographic region to region and the species and time of colonization must be developed for all areas using this type of evidence. Research has been conducted in British Columbia in a variety of habitats, seasons and geographic areas to develop a database for this Province
6. Drugs - the presence of drugs may affect the development of the insects. Work is planned to determine effects of common narcotics on insects in Canada[17].

Conclusion

In conclusion, insects are evidence! Forensic entomology is a very useful method of determining elapsed time since death after 72 hrs. It is accurate to a day or less, or a range of days, and may be the only method available to determine elapsed time since death. It is vital that the insects are collected properly and its accuracy depends on this and on suitable conditions for insects. There are some factors which are required to be considered before arriving at a conclusion, like the climate of the specific site where the body was found and this is to be correlated with the development of the insects. Seasonal variation should be taken into account and some standardization needs to be done so as to decrease the possibility of error in the estimation of the time of death.

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